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Several Different Enterovirus Serotypes Can Be Associated With Prediabetic Autoimmune Episodes and Onset of Overt IDDM

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In a prospective multicentre study described previously on prediabetic events in siblings of index cases with insulin-dependent diabetes mellitus, 31 children developed clinical diabetes during the observation period and 51 children seroconverted for islet cell antibodies or insulin autoantibodies. By using nonserotype specific EIA and RIA, it has shown recently that enterovirus infections in both groups were frequently associated with increases of islet cell antibody and/or insulin autoantibody titres. Serum specimens sequentially collected from 12 children during the prediabetic period were still available and were then tested for serotype-specific neutralizing antibodies. Plaque-neutralization assays were carried out for coxsackievirus A9, coxsackievirus B types 1 to 6, and echovirus types 1 and 11. An unequivocal monotypic increase in neutralizing antibodies was observed on seven occasions in six children, on one occasion with coxsackievirus A9, one with coxsackievirus B1, two with coxsackievirus B2, two with coxsackievirus B3, and one with coxsackievirus B5. In four patients, the infection was associated temporally with increases in the levels of islet cell antibodies, insulin autoantibodies and/or antibodies to glutamic acid decarboxylase, and in three other patients, it coincided with the clinical onset of insulin-dependent diabetes mellitus. These results suggest that the association of enterovirus infections with insulin-dependent diabetes mellitus is not restricted to serotype 4 of coxsackie B viruses suspected previously, but that several different serotypes might play a role in the pathogenesis of the disease. ***J. Med. Virol. 56:74–78, 1998.***

KEY WORDS: enterovirus, serotypes; enterovirus, antibodies; autoantigen, antibodies; insulin-dependent diabetes mellitus, risk factor; prediabetic period

INTRODUCTION

Enteroviruses have been implicated for many years in the pathogenesis of insulin-dependent diabetes mellitus (IDDM). Coxsackievirus B4 (CBV4) has been isolated from some cases with acute onset IDDM, and some of these isolates have been shown to be capable of inducing signs of a diabetes-like condition in mice [Yoon et al., 1979, 1990]. Elevated levels of enterovirus-specific IgM-class antibodies, a marker of recent or ongoing infection, have been observed frequently in pa-

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tients with newly diagnosed IDDM [King et al., 1983; Banatvala et al., 1985; Frisk et al., 1992; Dahlquist et al., 1995; Helfand et al., 1995]. There are also indications that maternal enterovirus infections during pregnancy will increase the risk of IDDM in the offspring [Dahlquist et al., 1995; Hyöty et al., 1995]. In a recent prospective cohort study, we used a wide spectrum of different cross-reactive enterovirus antibody tests and found that children who eventually progressed to clinical IDDM had suffered from enterovirus infections more frequently than control children remaining unaffected over the follow-up period [Hyöty et al., 1995]. Furthermore, enterovirus infections appeared to coincide with seroconversion to antibody positivity for islet cell antibodies (ICA) or insulin autoantibodies (IAA), or with the enhancement of the humoral IDDM-associated immune response [Hyöty et al., 1995; Hiltunen et al., 1997]. The children who converted to ICA positivity during an enterovirus infection more often had the high risk HLA-DQB1 genotype than subjects who remained ICA-negative [Hiltunen et al., 1997]. In previous serological studies, enterovirus infections were identified using EIA and RIA methods known to cross-react with different enteroviruses and thus not suitable for the identification of exact serotypes. To evaluate serotype identity of infections during prediabetic autoantibody responses, sequential serum samples from prediabetic siblings were tested for neutralizing antibodies to several different enteroviruses.

MATERIALS AND METHODS

Subjects and Sera

The population-based nationwide "Childhood Diabetes in Finland" (DiMe) Study included newly diagnosed diabetic index cases and a prospective cohort of nondiabetic siblings under the age of 20 years (n = 765) [Tuomilehto et al., 1992]. The follow-up comprised blood sampling every 3–6 months, starting from the time of diagnosis of IDDM in the index case. Altogether, 31 siblings contracted IDDM, and 51 initially nondiabetic and autoantibody-negative siblings became positive for ICA and/or IAA during the follow-up. Representative serum samples were available from 12 cases for the present study. Demographic data on the siblings studied are shown in Table I.

TABLE I. Demographic Data on Siblings Included in Present Study

Case no.	Sex	Age at diagnosis in index case (yr)	Age at IDDM diagnosis (yr)	Duration of follow-up (yr)
1	F	10.8	15.2	4.4
2	F	13.0		4.3
3	M	5.8	7.2	1.4
4	F	2.9	9.5	6.6
5	M	5.5	9.5	4.0
6	F	8.0	11.7	3.7
7	F	9.2	13.4	4.3
8	M	11.6	16.7	5.1
9	M	4.5	5.4	0.9
10	F	11.6	15.2	3.6
11	F	2.7	6.0	3.3
12	F	5.8	10.4	4.6

Cell Cultures and Virus Strains

GMK cells, a continuous cell line from African green monkey kidneys, were used for virus propagation. Eagle's minimal essential medium supplemented with 10% (for cell growth) or 1% (for virus propagation) fetal calf serum was used. The virus propagation medium also contained 20 mM Hepes (N-22-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), pH 7.4 and 20 mM $MgCl_2$.

The virus strains (CAV9, CBV1, CBV2, CBV3, CBV4, CBV5, CBV6, EV1, and EV11) used in the present study comprised reference strains of enteroviruses that were originally obtained from the American Type Culture Collection, kindly provided by Dr T. Hyypiä (Department of Virology, University of Turku, Finland). Crude virus preparations were cleared from cell debris by low-speed centrifugation and were used as such for the plaque neutralization assay. For preparation of the radioactively labelled virus, infected cells were labelled metabolically with ^{35}S-methionine and the virus was purified by sucrose gradient centrifugation as described by Abraham and Colonno [1984].

Neutralization

Serial fourfold dilutions of sera to be tested were mixed with an equal volume of pretitrated virus (~100 plaque-forming units in 6 μl). The mixture was incubated for 1 hour at 36°C and overnight at room temperature. Virus propagation medium (110 μl) was added, and the amount of virus still alive after neutralization was quantified by plaque assay.

Plaque Assay

Confluent GMK cell monolayers were grown in six-well plates (well diameter 3 cm, cat no. 9206, TPP, Switzerland) to a density of ~0.8×10^6 cells per well. The cells were washed once with Hanks balanced salt solution supplemented with 20 mM HEPES, pH 7.4 (h-Hanks). Virus samples preneutralized with tested serum were administered onto the cell monolayers in volumes of 50 μl. After 30 min incubation at 36°C, 2 ml of plaquing overlay (0.5% carboxymethyl cellulose in the virus propagation medium) was added. The amount of adsorbed infectious virus was measured by counting the plaques after 46 hr of incubation at 36°C. The reciprocal of the last dilution of serum able to block virus infectivity by 70% was taken as the titre of the serum specimen.

Assays of Autoantibodies

Islet cell antibodies (ICA) were determined using a standard immunofluorescence method using sections of frozen human group O pancreas [Bottazzo et al., 1974]. End point dilution titres were examined for the positive samples, and the results were expressed in JDF units relative to an international reference standard [Lern-

mark et al., 1991]. The detection limit was 2.5 JDF units. A tripling of the end point dilution titre was considered a significant increase in ICA level. Our laboratory has participitated in international workshops on the standardization of the ICA assay in which its sensitivity was 100%, specificity 98%, validity 98%, and consistency 98% in the fourth round.

Antibodies to the 65 kD isoform of glutamic acid decarboxylase were measured with a radioligand assay as described by Petersen et al. [1994]. Recombinant human islet GAD65 cDNA was transcribed and translated in vitro according to the manufacturer's instructions (Promega, Madison,WI. Serum samples (final dilution 1:25) were incubated overnight with ~30,000 cpm of ^{35}S- methionine-labelled in vitro translated human GAD65 in a total volume of 50 μl Tris-buffered saline with Tween (TBST). Protein-A-Sepharose (7.5 mg, Pharmacia, Uppsala, Sweden) in a total volume of 100 μl TBST was used to isolate the immunocomplexes. The quantity of precipitated immunocomplexes was counted with a scintillation counter. All the samples were analysed in quadruplicate with and without competition from an excess of unlabelled purified human recombinant GAD65 (1 mg/well) produced in baby hamster kidney cells. The results were expressed in relative units (RU), representing the specific binding as a percentage of that obtained with a positive standard serum. The cutoff limit for antibody positivity was set at the 99th percentile in 372 nondiabetic children and adolescents, i.e., at 6.6 RU. The intra-assay coefficient of variation was <5% and for the interassay, <10%. The disease sensitivity of the present assay was 80% and the specificity 94%, based on the 101 samples included in the second international GAD antibody (GADA) workshop [Schmidli et al., 1995]. An increase of 9.9 RU (3 SD-score in nondiabetic subjects) in the GADA level, was perceived as significant.

Insulin autoantibody (IAA) levels were analysed with a radiobinding assay modified from that described by Pamer et al. [1983]. Endogenous insulin was removed with acid charcoal prior to the assay, and free and bound insulin were separated after incubation with mono-^{125}I (Tyr A14)-human insulin (Novo Research Institute, Bagsvaerd, Denmark) for 20 hr in the absence or presence of an excess of unlabelled insulin. The IAA levels were expressed in nU/ml, where 1 nU/ml corresponds to a specific binding of 0.01% of the total counts. The interassay coefficient of variation was <8%. A subject was considered to be positive for IAA when the specific binding exceeded 54 nU/ml (99th percentile in 105 nondiabetic subjects). A significant increase was defined as a minimum of 33 nU/ml (3 SD-score in nondiabetic subjects).

Solid Phase Assay for Enterovirus Antibodies

Immunoglobulin class-specific antibodies were analysed by using a heavy-chain-capture RIA against a panel of enterovirus antigens including purified CBV4, CBV5, CAV9, echo1, and procapsid antigens of CBV3 and CBV5, as described previously [Frisk et al., 1989; Roivainen et al., 1993; Hyöty et al., 1995]. IgG-class antibodies were also analysed using an EIA method against a synthetic peptide antigen (amino acid sequence KEVPALTAVETGAT-C) derived from an immunodominant region of capsid protein VP1 [Roivainen et al., 1991], known to be a common antigenic determinant for several enteroviruses [Hovi and Roivainen, 1993]. The peptide EIA was found to be a specific test for detection of enterovirus infections [Samuelson et al., 1993].

RESULTS

Plaque-neutralizing antibodies to seven to nine enterovirus serotypes were measured in time-series of sera collected from 12 prediabetic children. Antibodies to other representatives of the 64 known serotypes could not be tested because only limited volumes of the serum samples were available. All children had neutralizing antibodies to at least one tested serotype. In one case, however, the level was only marginal. One child had antibodies to all nine virus strains examined, but usually the children were seropositive to a limited number of serotypes only (Table II). Antibodies to CAV9 were encountered most frequently (eight children), whereas antibodies to any of the other serotypes were found in three to five children.

Seroconversion to antibodies to one enterovirus examined was found on seven occasions in six children (Table III), whereas in the other children the antibody levels remained constant during the entire observation period. All seven enterovirus infections listed in Table III were associated with a concomitant increase in one or more categories of IDDM-associated autoantibodies or with the onset of clinical disease. In two patients, a CBV3 infection and in one case a CBV5 infection coincided with the clinical manifestation of IDDM. Concomitant increases in IAA and GADA levels were found in the child suffering from a CAV9 infection, and parallel ICA and GADA increases were observed in one case with a CBV2 infection (Fig 1., Case No.12). One child had two different enterovirus infections during the follow-up period: CBV2 infection was associated with parallel increases in ICA and IAA levels, and CBV1 infection with an increase in IAA level (Fig.1, Case No.4). Since the specimens tested were collected at 3- to 6-month intervals, and sometimes at an even longer interval, it was not possible to demonstrate unequivocally how close the coincidences were.

CONCLUSIONS

To evaluate serotype identity of enterovirus infections previously found to coincide with prediabetic autoantibody responses, serum specimens collected sequentially during the prediabetic period from 12 children were tested for serotype-specific neutralizing antibodies. An unequivocal monotypic increase in neutralizing antibodies was found on seven occasions in six children by using plaque-assay with some of the most common enteroviruses. In all these cases the infection was associated temporally with increases in IDDM-

TABLE II. Prevalence of Neutralizing Antibodies to Selected Enterovirus Serotypes in 12 Prediabetic Children*

Case no.	Serotype								
	CAV9	CBV1	CBV2	CBV3	CBV4	CBV5	CBV6	EV1	EV11
1	–	–	+	+	+	+	–	nt	nt
2	w	w	+	+	+	w	w	w	w
3	+	–	–	–	–	–	w	nt	nt
4	w	+	–	–	–	–	w	w	w
5	w	–	–	–	+	–	–	nt	nt
6	+	–	–	–	w	–	–	nt	nt
7	+	–	–	+	–	+	–	nt	nt
8	+	–	–	–	–	–	w	–	+
9	–	–	–	–	–	–	–	–	w
10	–	nt	–	+	–	–	–	nt	nt
11	–	w	–	+	–	–	w	–	+
12	+	w	+	–	–	–	–	+	–

*nt, not tested; –, no detectable antibodies in any tested specimen; w, antibodies detected in one or more specimens at the detection limit level only; +, definite seropositivity.

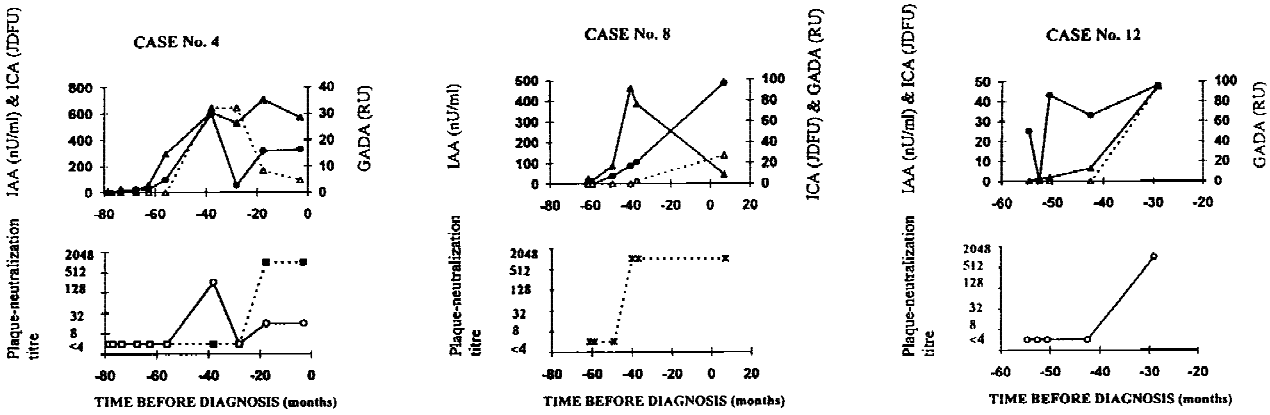

Fig. 1. Association of enterovirus infections with increases in IDDM-associated antibodies in three prediabetic children. Serum specimens collected sequentially during the prediabetic phase were tested for antibodies to IAA (insulin autoantibodies, ●), ICA (islet cell antibodies, △), and GADA (glutamic acid decarboxylase antibodies, ▲). Neutralizing enterovirus-specific antibodies were measured using plaque reduction assay, and the results obtained with CBV1 (■), CBV2 (○) and CAV-9 (*) are shown in the figure.

TABLE III. Serologically Defined Enterovirus Infections in Studied Children*

Case no	Serotype	Antibody titres	Concomitant increase in			Coinciding presentation of IDDM
			ICA	IAA	GADA	
4	CBV2	<4–256	+	+	–	–
4	CBV1	4–1024	–	+	–	–
7	CBV5	<8–256	NT	NT	NT	+
8	CAV9	<4–1024	–	+	+	–
10	CBV3	<8–64	–	NT	–	+
11	CBV3	<4–256	–	–	–	+
12	CBV2	<4–1024	+	–	+	–

*NT, not tested.

associated autoantibodies or the onset of clinical disease. The relatively low incidence of enterovirus infections might appear surprising, since they are considered to be common in this age group, and especially since it was observed previously that enterovirus infections were more common in this study cohort than in control children [Hyöty et al., 1995]. However, now we tested antibodies to only nine of 64 enterovirus serotypes known to be pathogenic in humans, and corresponding serum specimens from control children were not evaluated. Furthermore, it is possible that infections due to prime strains or other antigenic variants may not be detected by a neutralization assay using the prototype strains. It is worth noting that none of the type-identified infections was caused by coxsackievirus B4, the serotype traditionally referred to as "diabetogenic."

Two out of seven infections, caused by CBV-1 and CBV-5, respectively, and documented by the current assays, were not detected by solid-phase antibody as-

says (RIA and EIA) employed previously using a wide spectrum of cross-reacting enterovirus antigens [Hyöty et al., 1995]. This suggests that the sensitivity of the set of our solid-phase antibody tests is not complete, perhaps because cross-reacting antibody responses may depend on the host's previous infection history or on other host-dependent factors, as well as antigenic variation in the epidemic virus strains. Similarly, some of the responses detected in our previous assays (results not shown) could not be confirmed by the current neutralization assay. This is not surprising because of the limited number of serotypes examined.

In conclusion, it was shown that infections caused by several different enteroviruses can be associated with IDDM, even though only a small group of common enteroviruses was used for the serum neutralization assay. The mechanisms by which enteroviruses induce or accelerate the process, eventually resulting in clinical IDDM have not been defined so far, although different mechanisms have been discussed [See and Tilles, 1995]. Nor is it known whether enterovirus serotypes and/or strains within a given serotype differ in their diabetogenic potential, or whether certain genetic determinants of the virus are more important than antigenic structure with respect to diabetogenic potential [Kang et al., 1994; Titchener et al., 1994]. In the case of another diabetogenic picornavirus, the murine encephalomyocarditis virus, the development of diabetes in infected mice is related to a single amino acid change in a virus protein [Bae et al., 1993].

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REFERENCES

Abraham G, Colonno RJ (1984): Many rhinovirus serotypes share the same cellular receptor. Journal of Virology 51:340–345.

Bae YS, Yoon JW (1993): Determination of diabetogenicity attributable to a single amino acid, Ala776, on the polyprotein of encephalomyocarditis virus. Diabetes 42:435–443.

Banatvala JE, Bryant J, Schernthaner G, Borkenstein M, Schober E, Brown D, De Silva LM, Menser MA, Silink M (1985): Coxsackie B, mumps, rubella and cytomegalovirus specific IgM responses in patients with juvenile-onset insulin-dependent diabetes mellitus in Britain, Austria, and Australia. Lancet 1:1409–1412.

Bottazzo GF, Florin-Christensen A, Doniach D (1974): Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet II:1279–1282.

Dahlquist G, Ivarsson S, Lindberg B, Forsgren M (1995): Maternal enteroviral infection during pregnancy as a risk factor for childhood IDDM. A population-based case-control study. Diabetes 44: 408–413, 1995.

Frisk G, Friman G, Tuvemo T, Fohlman J, Diderholm H (1989): Enterovirus IgM detection: Specificity of u-antibody-capture radioimmunoassays using virions and procapsids of Coxsackie B virus. Journal of Virological Methods 24:191–202.

Frisk G, Nilsson E, Tuvemo T, Friman G, Diderholm H (1992): The possible role of Coxsackie A and Echo viruses in the pathogenesis of type 1 diabetes mellitus studied by IgM analysis. Journal of Infectious Diseases 24:13–22.

Helfand RF, Gary HE, Jr., Freeman CY, Anderson LJ, Pittsburgh Diabetes Research Group and Pallansch MA (1995): Serological evidence of an association between enteroviruses and the onset of type 1 diabetes mellitus. Journal of Infectious Diseases 172:1206–1211.

Hiltunen M, Hyöty M, Knip M, Ilonen J, Reijonen H, Vähäsalo P, Roivainen M, Leinikki P, Hovi T, Åkerblom HK, the Childhood Diabetes in Finland (DiMe) Study Group (1997): ICA seroconversion in children is temporally associated with enterovirus infections. Journal of Infectious Diseases 175: 554–6

Hovi T, Roivainen M (1993): Peptide antisera targeted to a conserved sequence in poliovirus capsid protein VP1 cross-react widely with members of the genus Enterovirus. Journal of Clinical Microbiology 31:1083–1087.

Hyöty H, Hiltunen M, Knip M, Laakkonen M, Vähäsalo P, Karjalainen J, Koskela P, Roivainen M, Leinikki P, Hovi T, Åkerblom HK, the Childhood Diabetes in Finland (DiMe) Study Group (1995): A prospective study on the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Diabetes 44: 652–657.

Kang Y, Chatterjee NK, Nodwell MJ, Yoon JW (1994): Complete nucleotide sequence of a strain of coxsackie B4 virus of human origin that induces diabetes in mice and its comparison with nondiabetogenic coxsackie B4 JBV strain. Journal of Medical Virology 44:353–361.

King ML, Shaikh A, Bidwell D, Voller A, Banatvala JE (1983): Coxsackie-B-virus-specific IgM responses in children with insulin-dependent (juvenile-onset;type 1) diabetes mellitus. Lancet 1: 1397–1399.

Lernmark Å, Molenaar JL, Van Beers WA, Yamaguchi Y, Nagataki S, Ludvigsson J, Maclaren NK (1991): The Fourth International Serum Exchange Workshop to standardize cytoplasmic islet cell antibodies. Diabetologia 34:534–535.

Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL (1983): Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 222:1337–1339.

Petersen JS, Hejnaes KR, Moody HA, Karlsen AE, Marshall MO, Hoier-Madsen M, Boel E, Michelsen BK, Dyrberg T (1994): Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. Diabetes 43:459–467.

Roivainen M, Närvänen A, Korkolainen M, Huhtala M-L, Hovi T (1991): Antigenic regions of poliovirus type 3/Sabin capsid proteins recognized by human sera in the peptide scanning technique. Virology 180:99–107.

Roivainen M, Agboatwalla M, Stenvik M, Rysä T, Akram DS, Hovi T (1993): Intrathecal immune response and virus-specific immunoglobulin M antibodies in laboratory diagnosis of acute poliomyelitis. Journal of Clinical Microbiology 31:2427–2432.

Samuelson A, Glimåker M, Skoog E, Cello J, Forsgren M (1993): Diagnosis of enteroviral meningitis with IgG-EIA using heat treated virions and synthetic peptides as antigens. Journal of Medical Virology 40:271–277.

Schmidli RS, Colman PG, Bonifacio E and participating laboratories (1995): Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies. The Second International GADAb Workshop. Diabetes 44:636–640.

See DM, Tilles JG: Pathogenesis of virus-induced diabetes in mice (1995). Journal of Infectious Diseases 171:1131–8.

Titchener PA, Jenkins O, Szopa TM, Taylor KW, Almond JW (1994): Complete nucleotide sequence of a beta-cell tropic variant of coxsackievirus B4. Journal of Medical Virology 42:369–373.

Tuomilehto J, Lounamaa R, Tuomilehto-Wolf E, Reunanen A, Virtala E, Kaprio EA,Åkerblom HK (1992): Epidemiology of childhood diabetes in Finland - background of a nationwide study of Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 35:70–76.

Yoon J-W, Austin M, Onodera T, Notkins AL (1979): Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. New England Journal of Medicine 300:1173–1179.

Yoon J-W (1990): The role of viruses and environmental factors in the induction of diabetes. Current Topics of Microbiological Immunology 164:95–123.